

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/IL05/000039

International filing date: 12 January 2005 (12.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US  
Number: 60/536,852  
Filing date: 14 January 2004 (14.01.2004)

Date of receipt at the International Bureau: 25 February 2005 (25.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

15 FEB 2005

PA 1276801



# THE UNITED STATES OF AMERICA

**TO ALL TO WHOM THESE PRESENTS SHALL COME:**

**UNITED STATES DEPARTMENT OF COMMERCE**

**United States Patent and Trademark Office**

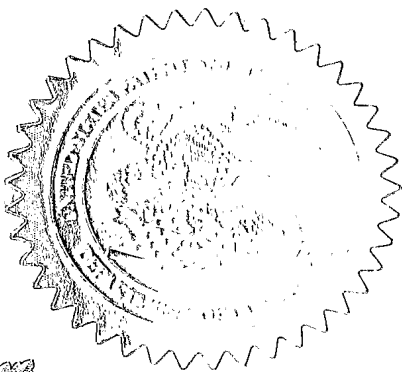
**January 28, 2005**

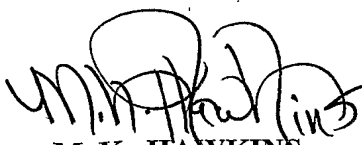
**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM  
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK  
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT  
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A  
FILING DATE UNDER 35 USC 111.**

**APPLICATION NUMBER: 60/536,852**

**FILING DATE: *January 14, 2004***

**By Authority of the  
COMMISSIONER OF PATENTS AND TRADEMARKS**



  
**M. K. HAWKINS**  
**Certifying Officer**

Please type a plus sign (+) inside this box → ☐

PTO/SB/16 (8-00)

Approved for use through 10/31/2002. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

## PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)					
Given Name (first and middle (if any))		Family Name or Surname		Residence (City and either State or Foreign Country)	
Ayala		BARAK		11 Be'eri Street Tel Aviv Israel	
<input type="checkbox"/> Additional inventors are being named on the ____ separately numbered sheets attached hereto.					
TITLE OF THE INVENTION (280 characters max)					
BIOCIDES METHOD AND APPARATUS					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number		<input type="text"/>		<div>Place Customer Number Bar Code Label here</div>	
OR Type Customer Number here					
<input checked="" type="checkbox"/> Firm or Individual Name		ABELMAN, FRAYNE & SCHWAB Attorneys at Law			
Address		150 East 42 <sup>nd</sup> Street			
Address		New York, New York 10017			
City		State		Zip	
Country		U.S.A.	Telephone	(212) 949-9022	Fax (212) 949-9190
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification (includes drawings)		Number of Pages		<input type="checkbox"/> CD(s), Number	
		32			
<input checked="" type="checkbox"/> Drawing(s)		Number of sheets		<input type="checkbox"/> Other (specify)	
		5			
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.					
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number:		01-0035		FILING FEE AMOUNT (\$)	
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.				\$80.00	
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME

TELEPHONE

(212) 949-9022

Date

January 14, 2004

REGISTRATION NO.

24,156

(if appropriate)

Docket Number:

206,419

### USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you req reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED Stop Provisional Application, Assistant Commissioner for Patents Alexandria, VA 22313-1450.

"EXPRESS MAIL" Label No.: ET537601333US Date of Deposited: January 14, 2004 This correspondence is being Deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10 on the date indicated above and addressed to: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

### Field of the Invention

The invention relates to method and apparatus for inhibiting the growth of living organisms.

### Background

U.S. Patents Nos. 5,795,487, 5,976,386, 6,110,387, 6,132,628, 6,429,181, 6,478,972, and 6,533,958 and published U.S. Patent Application No. 20030121868, the contents of all of which are incorporated herein by reference, are believed to represent relevant prior art.

### Brief Description of the Drawings

Embodiments of the invention are more particularly described with respect to a number of examples set forth below, and also with respect to the accompanying drawings wherein:

FIG. 1 is a block diagram illustrating one form of apparatus constructed and operative to enable the practice of an embodiment of the present invention;

FIG. 2 is a similar block diagram illustrating another apparatus constructed and operative to enable the practice of an embodiment of the present invention;

FIG. 3 is a similar block diagram illustrating another apparatus constructed and operative to enable the practice of an embodiment of the present invention;

FIG. 4 is a similar block diagram illustrating another apparatus constructed and operative to enable the practice of an embodiment of the present invention; and

FIG. 5 is a similar block diagram illustrating another apparatus constructed and operative to enable the practice of an embodiment of the present invention.

The apparatus illustrated in FIG. 1 produces a biocide that is introduced into a liquid 3, such as water, at a location 2. The biocide is formed by mixing *in situ* two solutions, a first solution comprising a hypochlorite oxidant solution within a reservoir 4, and a second solution comprising at least one ammonium salt and optionally, bromide, within a reservoir 6.

As shown in Fig. 1, water, e.g., tap water, de-ionized water, soft water, or industrial water, is fed from a source 8 via a water pipe 10 through a pair of branch lines 12, 14, connected in parallel to each other, to a mixer 21 which feeds common outlet pipe 16 leading to the liquid 3 at the location 2. Each of the two parallel branch lines 12, 14, includes a venturi tube 18, 20 having an inlet port 18a, 20a, connected in the respective branch line 12, 14, and an outlet port 18b, 20b, connected to mixer 21 which connects to the common outlet line 16 leading to the liquid to be treated. Each of the venturi tubes 18, 20, includes a third port 18c, 20c, leading to the reservoir 4, 6, of the respective solution to be added to the water flowing through the outlet line 16.

The two venturi tubes 18, 20, thus constitute dosing pumps which continuously and synchronously inject both hypochlorite oxidant solution from reservoir 4, and the solution comprising at least one ammonium salt (and, optionally, bromide) from reservoir 6, into the water from source 8 in proportions which are predetermined for

optimal formation of the biocide. The two solutions are thus diluted to form first and second dilutions, respectively, and the first and second dilutions are mixed in mixer 21 and react with each other in mixer 21 which feeds into outlet pipe 16, so that the reaction product, namely the biocide produced by the reaction of these two dilutions, is introduced as it is produced *in situ* into the liquid 3.

The two branch lines 12, 14 for the two venturi tubes 18, 20 include control valves 22, 24, which enable the flow rate of the water to be controlled via the two venturi tubes 18, 20. Lines 26, 28 connecting the two reservoirs 4,6 to their respective venturi tubes 18, 20 also include valves, shown at 30, 32, for controlling the dosage of the chemicals into the water passing through the venturi tubes. The latter valves also enable the supply of chemicals to be terminated at the end of the introduction of the biocide, so that continued flow of the water via the branch lines 12, 14, mixer 21 and the outlet line 16 will wash away any residue of these chemicals, or their decomposition products, and thereby avoid accumulation of decomposition products which may form at the end of each biocide production cycle in the outlet line 16 or in mixer 21.

The control of the foregoing valves is done by a control system, schematically illustrated by block 40. Outlet line 16, therefore, may also include a pH sensor 47 for sensing the pH of the biocide, and controlling the control system 40 in response thereto.

Control system 40 also controls the supply of the water from source 8 via an electrical valve 48. Control system 40 can further control an alarm 50 or other signalling device. The illustrated system may further include a timer 52 which is presettable to fix both the lengths of time for which the biocide is to be fed via the outlet line 16 to the liquid to be treated, as well as the time intervals between such feedings of the biocide. Control system 40 may also be operative to control the operation of mixer 21.

The water supply line 10 from the water source 8 to the two branch lines 12, 14, may include additional control devices. For purposes of illustration, the accompanying drawings schematically illustrate the following additional control devices: a manual control valve 53, enabling manual control of the water flow from the source 8; a pressure reducer 54 for reducing the pressure from the source; a pressure sensor 56 which may also be used as an input into the control system 40; a flow meter 58 for indicating the flow rate or flow volume; a pressure gauge 60 for indicating the pressure in line 10; a pressure relief valve 62; and a one-way valve 64.

In one embodiment of the invention, the two venturi tubes 18, 20, and their controls, are designed so as to synchronously feed the same volumes of solutions from the two sources 4, 6 even though the viscosities of the two solutions may be different. The illustrated system may operate at a constant predetermined water pressure and at a constant ratio of predetermined dilution of the two solutions to the water passing via the branch lines 12, 14, through the two venturi tubes 18, 20. Each of these parameters can be controlled as described above so that the solutions from the two sources 4, 6, are simultaneously and synchronously injected in the desired predetermined proportions with respect to each other, and also with respect to the water flowing through the venturi tubes 18, 20 from the source 8. In another

embodiment of the invention, the volumes of solutions from sources 4 and 6 may be different, provided that the final molar ratio of oxidant, ammonium salt and, when present, bromide, is within a predetermined range.

As indicated earlier, the solution in reservoir 4 comprises a hypochlorite oxidant, and the solution within reservoir 6 comprises at least one ammonium salt and optionally bromide. The bromide may be provided in any suitable form. In an embodiment of the invention, the bromide is provided as an alkali or alkaline earth metal bromide salt, such as lithium bromide, sodium bromide, potassium bromide, calcium bromide, magnesium bromide or hydrobromic acid. The at least one ammonium salt is selected from the group of ammonium salts which are acid salts of ammonia and an organic or inorganic acid which contains a primary or secondary amine group. Thus, the ammonium salt may be a salt formed by  $\text{NH}_3$  and a compound such as proline or a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

- H,
- COOH,
- COOR<sup>3</sup> wherein  $\text{R}^3$  is selected from the group consisting of  
C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,
- CHR<sup>4</sup>COOH wherein  $\text{R}^4$  is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
-COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,
- COONHR<sup>5</sup> wherein  $\text{R}^5$  is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,
- C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein  $\text{R}^6$  is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
-OH, -COOH, or -NH<sub>2</sub>,
- SO<sub>3</sub>H,
- SO<sub>2</sub>R<sup>7</sup> wherein  $\text{R}^7$  is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,
- OH,
- C<sub>1-8</sub> alkyl,
- C<sub>1-8</sub> alkyl optionally substituted by one or more of:  
-OH, -COOH, -NH<sub>2</sub> or =NH,
- NHR<sup>7</sup>, wherein  $\text{R}^7$  is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,

provided that  $\text{R}^1$  and  $\text{R}^2$  are not both H,

and provided that at least one of  $\text{R}^1$  and  $\text{R}^2$  contains a group -COOH or -SO<sub>3</sub>H.

The oxidant is chosen from alkali and alkaline earth metal hypochlorites, e.g. lithium hypochlorite, sodium hypochlorite, potassium hypochlorite, calcium hypochlorite or magnesium hypochlorite.

In an embodiment of the invention, the biocide has a pH of at least 8.0 just prior to its injection into liquid 3. In another embodiment of the invention, the biocide has a pH of at least 9.5 just prior to its injection into liquid 3. In an embodiment of the

invention, the biocide has a pH of not more than 11.5 just prior to its injection into liquid 3. In an embodiment of the invention, the biocide is injected at a rate to maintain in the biocide a stable pH of at least 8.0 as it is produced.

FIG. 2 illustrates another apparatus, constructed and operative to provide a biocide in accordance with an embodiment of the invention. The apparatus shown in FIG. 2 is similar to that in FIG. 1, with like numbers denoting elements of the system of FIG. 2 which are the same as in the system of FIG. 1 and which operate in the same way. The principal difference between the two systems is that in the system of FIG. 2, the venturi tubes 18, 20 are replaced by pulsatile pumps  $P_1$ ,  $P_2$ . The two pulsatile pumps  $P_1$ ,  $P_2$  are also controlled by the control system 40 so as to synchronously meter the liquids from the two reservoirs 4, 6, via feed lines 26, 28, in a manner similar to that of the venturi tubes 18, 20, in the system described above with respect to FIG. 1, except that the liquid pumped out of pumps  $P_1$  and  $P_2$  is mixed with the water in branch lines 12, 14 at mixers  $M_1$ ,  $M_2$  as the water in branch lines 12, 14 flows to mixer 21 and then to outlet line 16. Pulsatile pumps  $P_1$  and  $P_2$  may be replaced by other types of pumps, such as peristaltic pumps and the like.

It will be appreciated that although Figs. 1 and 2 depict embodiments of the invention in which the reservoir 6 contains a solution comprising at least one ammonium salt and optionally bromide, when bromide is used the dilution containing bromide and ammonium salt need not be prepared from a solution containing bromide and ammonium salt which is stored in a reservoir, and the dilution may be prepared immediately prior to being mixed with the hypochlorite oxidant. It will also be appreciated that the first and second solutions may be sufficiently dilute that little or no additional dilution is necessary prior to mixing in mixer 21.

FIG. 3 illustrates another apparatus, constructed and operative to provide a biocide in accordance with an embodiment of the invention. The apparatus shown in FIG. 3 is similar to that in FIG. 2, with like numbers denoting elements of the system of FIG. 3 which are the same as in the system of FIG. 2 and which operate in the same way. In the system of FIG. 3, reservoir 6 contains a solution of at least one ammonium salt which is substantially free of bromide. Solution containing bromide is stored in reservoir 6'. Bromide is thus pumped by pump  $P_3$  through feed line 28' to liquid pumped out of  $P_2$  and is mixed with the water in branch line 14 at mixer  $M_3$  as the water in branch line 14, which has already been mixed with ammonium salt-containing solution from reservoir 6, flows to mixer 21 and then to outlet line 16. Pulsatile pump  $P_3$ , like pumps  $P_1$ ,  $P_2$ , is controlled by the control system 40 so as to synchronously meter the liquid from reservoir 6' via feed line 28'.

FIG. 4 illustrates another apparatus, constructed and operative to provide a biocide in accordance with an embodiment of the invention. The apparatus shown in FIG. 4 is similar to that in FIG. 3, with like numbers denoting elements of the system of FIG. 4 which are the same as in the system of FIG. 3 and which operate in the same way. The principal difference between the two systems is that in the system of FIG. 4, pulsatile pump  $P_3$  is connected to a third branch line 15. Bromide is thus pumped by pump  $P_3$  through feed line 28' to mixer  $M_3$ , where it is mixed with the water in branch line 15 as the water in branch line 15 flows to mixer 21 and then to outlet line 16.

FIG. 5 illustrates another apparatus, constructed and operative to provide a biocide in accordance with an embodiment of the invention. The apparatus shown in FIG. 5 is similar to that in FIG. 4, with like numbers denoting elements of the system of FIG. 5 which are the same as in the system of FIG. 4 and which operate in the same way. The principal difference between the two systems is that in the system of FIG. 3, water exiting mixer M<sub>3</sub> flows not to mixer 21 but directly to outlet line 16, where it it mixes with the water exiting mixer 21. Optionally, an additional mixer 21' may be employed to mix the liquid flowing from mixer M<sub>3</sub> with the liquid exiting mixer 21 before the liquid exits outlet line 16.

In the context of this patent application, the term "effective", when used in reference to a biocide, means that the biocide is capable of controlling microbial growth, as evidenced by the ability to kill at least 50% of the microorganism in a liquid test sample within 3 hours after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.

The present invention will be better understood through the following illustrative and non-limitative examples of embodiments thereof.

#### **Experimental:**

General: Tests were conducted in an aqueous test system consisting in each instance deionized (DI) water to which was added starch (~7.5 g/l), calcium hydroxide (94 ppm), and sodium bicarbonate (1320 ppm); pH was adjusted to 8.17 using hydrochloric acid. A suspension of microorganisms was prepared from a sample of pink slime removed from the surface of a paper machine and added to the DI water. Microorganisms were grown at 37°C.

As controls, in each test (a) biocide was added to DI water only, and (b) a sample of medium was left untreated by biocide.

In the following examples, solutions of the active biocidal ingredient in accordance with embodiments of the present invention were prepared by using apparatus providing a continuous, synchronous production of the active biocidal ingredient as described above. In those tests where biocide was prepared from amine and hypochlorite only, the procedure described above was followed, but the mixture containing amine and bromide mixture was replaced by an amine or amine solution only. The decomposition rate of the concentrated active ingredient was monitored in the examples below by measuring the residue of total chlorine in the concentrate.

#### **Example 1: Oxidation Reduction Potential (ORP).**

Using a pair of electrodes, oxidation-reduction potentials were measured in accordance with G. Degramont, "Water Treatment Handbook", Springer-Verlag, 1991, pp. 249-250, the contents of which are incorporated herein by reference. Because the oxidation-reduction potential depends on the activity of the oxidized and reduced forms of a substance, different substances can be classified by comparing their ORP's.

In this example, four tests were conducted:



Test 1: In accordance with U.S. Patent No. 6,478,972 ("Shim"), sodium sulfamate (14.62 g sulfamic acid dissolved in 100 ml DI water containing 7.2 g NaOH) and sodium hypochlorite (10.5% w/v in 100 ml DI water, expressed as  $\text{Cl}_2$ ) were mixed (molar ratio of sulfamate to  $\text{Cl}_2$  1.007 : 1) to produce what Shim terms a "stabilized hypochlorite solution".

Test 2: In accordance with Shim, sodium sulfamate (14.62 g sulfamic acid dissolved in 100 ml DI water containing 7.2 g NaOH) and sodium hypochlorite (10.5% w/v in 100 ml DI water, expressed as  $\text{Cl}_2$ ) were mixed (molar ratio of sulfamate to  $\text{Cl}_2$  1.007 : 1) to produce what Shim terms a "stabilized hypochlorite solution". Sodium bromide (15.5% w/v) (molar ratio of  $\text{Br}^-$  to  $\text{Cl}_2$  1.014 : 1) was mixed into the "stabilized hypochlorite solution". A significant change in color was noticed as soon as NaBr was added to the "stabilized hypochlorite concentrate". The resulting mixture was immediately added to each of the aqueous test systems, in defined volumes to maintain feed levels of 2.5, 5.0 and 7.5 ppm (expressed as total chlorine) respectively.

Test 3: Sodium bromide (15.5% w/v) was mixed into a "stabilized hypochlorite solution" (molar ratio of  $\text{Br}^-$  to  $\text{Cl}_2$  1.014 : 1) prepared as per Test 1. A slight change in color was noticed as soon as NaBr was added to the "stabilized hypochlorite solution". The resulting mixture was immediately added to each of the aqueous test systems, in defined volumes to maintain feed levels of 2.5, 5.0 and 7.5 ppm (expressed as total chlorine) respectively.

Test 4: In accordance with Shim, sulfamic acid (14.62 g in 100 ml DI water) and sodium hypochlorite (10.5% w/v in 100 ml DI water, expressed as  $\text{Cl}_2$ ) were mixed. The mixture was immediately added to each of the aqueous test systems, in defined volumes to maintain feed levels of 4.2, 8.4 and 12.6 ppm (expressed as total chlorine) respectively. NaBr (15.5% w/v, molar ratio of  $\text{Br}^-$  to  $\text{Cl}_2$  1.014 : 1) was simultaneously added separately to the aqueous system.

In tests 2, 3 and 4, ORP was measured two hours after the biocide was added to the aqueous system. The results are presented in Table 1, where ppm refers to the biocide concentration, expressed as  $\text{Cl}_2$ , immediately after addition to the test sample:

Table 1

treatment	ORP (volts)		
	test 4	test 2	test 3
8.4 ppm, DI only	340	405	420
4.2 ppm, medium	238	310	348
8.4 ppm, medium	231	294	330
12.6 ppm, medium	250	284	295
0 ppm, medium	200	200	200

The results in Table 1 show that the order and mode of addition of the chemicals in the method of Shim is significant, as is the identity of the chemicals.

### Example 2: Residual Total Chlorine

Residual total chlorine was measured in the aqueous system 10 minutes after addition of the biocide, and again after 24 hours. As is known in the art, the rate of degradation of an oxidizer in an aqueous system is system-specific, i.e. the degradation rate of a given oxidizer is reproducible in a given aqueous system.

Test 4 is the same Test 4 conducted in Example 1.

Test 5: Sodium sulfamate (14.62 g sulfamic acid dissolved in 100 ml DI water containing 7.2 g NaOH) was mixed with NaBr (15.5 g in 100 ml DI water) (sodium sulfamate and NaBr both equimolar to sodium hypochlorite) and diluted in DI water. Sodium hypochlorite (10.5% w/v in 100 ml DI water, expressed as  $\text{Cl}_2$ ) was diluted in DI water (to a concentration of 4200 ppm, 0.42% w/v expressed as  $\text{Cl}_2$ , equimolar to sulfamate and to bromide ion). The two diluted solutions were mixed according to the procedure described above. The biocide was immediately added to the aqueous system. The results are presented in Table 2 (presented as total chlorine as percent of feed).

Table 2

treatment	Total $\text{Cl}_2$ (as % of feed)			
	test 4 - 10 min	test 4 - 24 hours	test 5 - 10 min	test 5 - 24 hours
8.4 ppm, DI	48.8	53.6		
4.2 ppm, DI			119.05	107.1
2.1 ppm			42.86	2.4
4.2 ppm	31	19.05	57.14	50
6.3 ppm			71.4	57.1
8.4 ppm	29.8	27.4		
12.6 ppm	39.7	34.1		

\*In the control samples in which biocide treatment was 0 ppm, the total  $\text{Cl}_2$  was 0 ppm after both 10 minutes and 24 hours.

These results show that biocide formed according to the Shim et al. is different than biocide formed by diluting and mixing the ingredients prior to injection into the aqueous system to be treated.

### Example 3: Adenosine triphosphate (ATP) concentration

ATP levels serve as a measure for the biochemical activity of aerobic microorganisms, and as such serve as a good model for the viability of a microbial culture after it has been exposed to a biocide. Thus, in the aqueous system of Tests 4 and 5 described above, the concentration of ATP was measured, 20 minutes after the addition of the biocide. The results are presented in Tables 3 and 3A (in relative units (rlu) and ng/ml, respectively).

Table 3

	test 4	test 5
treatment	ATP (rlu)	ATP (rlu)
54.2ppm, DI only	0	0
8.4 ppm, DI only	0	0
2.1 ppm, medium		1850
4.2 ppm, medium	2400	1700
6.3 ppm, medium		1400
8.4 ppm, medium	2250	
12.6 ppm, medium	1800	
0 ppm, medium	1950	1950

"rlu" = relative units

Table 3A

	test 4	test 5
treatment	ATP (ng/ml)	ATP (ng/ml)
8.4ppm,DI		
4.2ppm DI		
2.1ppm		0.58
4.2ppm	0.75	0.53
6.3 ppm		0.44
8.4 ppm	0.7	
12.6 ppm	0.56	
0 ppm, medium	0.61	

The results presented in Tables 3 and 3A show that after a contact time of 20 minutes, the biocide produced according to the procedure of Shim et al. (sodium hypochlorite stabilized with sulfamic acid added to water to be treated, then NaBr added thereafter to the water to be treated) is less effective in controlling microbial activity than the biocide produced from mixing dilute sodium sulfamate, dilute sodium bromide and dilute sodium hypochlorite. This result is in accordance with the data presented by Shim, who states that antimicrobial efficacy of his product occurs only 24 hours or more after administration to the water to be treated.

#### Example 4: Total aerobic counts.

Test 6: A biocide was prepared by diluting sodium sulfamate (14.62 g in 100 ml DI water containing 7.2 g NaOH, 5850 ppm) in a first stream of DI water, diluting sodium hypochlorite in a second stream of DI water (4600 ppm, 0.46% w/v), mixing the two dilutions in a conduit and immediately adding the aqueous system to be treated, as described above.

The results of viable counts of aerobic MO's in Tests 5 and 6 are presented in Tables 4 and 4A.

Table 4

treatment	test 6	test 5
dosage, Cl <sub>2</sub>	aerobic cfu/ml	aerobic cfu/ml
2.1 ppm	$1.30 \times 10^5$	$5.86 \times 10^4$
0 ppm	$1.30 \times 10^5$	$1.30 \times 10^5$

cfu = colony forming units

Table 4A

treatment	test 6	test 5
dosage, Cl <sub>2</sub>	aerobic cfu/ml (% kill)	aerobic cfu/ml (% kill)
2.1 ppm	0%	55%

The results in Table 4 demonstrate that producing a biocide by first producing a dilute mixture of bromide and sulfamate, then mixing this mixture with dilute hypochlorite and injecting the product into the liquid to be treated, while ensuring that there is no excess oxidant (hypochlorite) during the production of the biocide, yields a more efficacious biocide than does mixing a dilute sulfamate with dilute hypochlorite and injecting the product into the liquid to be treated.

Example 6: viable counts in media containing high sugars.

10-fold serial dilutions of each of the aqueous system test samples in sterile saline containing sodium thiosulfate was conducted 30 minutes after the biocide was added to the aqueous systems; the resulting serially ten fold diluted solutions were mixed in molten high sugar Agar. Viable counts of the colonies in the agar were counted after 48 hours, and are presented in Table 6 as cfu/ml.

Test 9: A biocide was prepared by diluting guanidinium sulfate in a first stream of DI water (0.647 g guanidinium sulfate (MW 216.22) in 100 ml DI water), diluting sodium hypochlorite in a second stream of 100 ml DI water (to a concentration of 4200 ppm, 0.42% w/v expressed as Cl<sub>2</sub>), mixing the two dilutions in a conduit and immediately adding the mixed dilutions to the aqueous system to be treated, as described above.

Test 10: A biocide was prepared by mixing guanidinium sulfate (0.647 g) with NaBr (0.62 g, NaBr equimolar to sodium hypochlorite) and diluting in a first stream of 100 ml DI water, diluting sodium hypochlorite in a second stream of 100 ml DI water (to a concentration of 4200 ppm, 0.42% w/v expressed as Cl<sub>2</sub>), mixing the two dilutions in a conduit and immediately adding the mixed dilutions to the aqueous system to be treated. The results are shown in Table 6, which shows the number of sugar-consuming colony forming units (cfu), and Table 6A, which present the same data as % survival relative to the non-biocide treated control.

Table 6

treatment	test 9	test 10
	sugar cfu/ml	sugar cfu/ml
4.2 ppm, DI only	0	0
2.1 ppm, medium	$9.20 \times 10^2$	$3.30 \times 10^2$
4.2 ppm, medium	$9.80 \times 10^2$	$4.00 \times 10$
6.3 ppm, medium	$8.00 \times 10$	$5.00 \times 10$
0 ppm, medium	$1.06 \times 10^4$	$1.06 \times 10^4$

Table 6/A

treatment	test 9	test 10
	sugar cfu/ml % survival	sugar cfu/ml %survival
2.5 ppm, medium	8.68	3.11
5 ppm, medium	9.25	0.38
7.5 ppm, medium	0.75	0.42
0 ppm, medium	100.00	100.00

The results in Tables 6 and 6A demonstrate that producing a biocide by first producing a dilute mixture of bromide and guanidinium sulfate, then mixing this mixture with dilute hypochlorite and injecting the product into the liquid to be treated, while ensuring ensuring that there is no excess oxidant (hypochlorite) during the production of the biocide, yields a more efficacious biocide than does mixing dilute guanidinium sulfate with dilute hypochlorite and injecting the product into the liquid to be treated.

#### Example 7: Efficiency of production of the biocide

Residual total chlorine in DI-only control samples was measured 10 minutes and 20 hours after addition of biocide, using the DPD colorimetric method (see "Standard Methods for Examination of Waste and Waste Water", 17th Edition (1989), pp. 4-62 to 4-64), the contents of which are incorporated herein by reference.

Total chlorine was measured for the control tests in DI water-only samples of Tests 1-6 described above. The results are presented in Table 7.

Table 7

	%Cl <sub>2</sub> - 10 min	%Cl <sub>2</sub> - 20 Hours
test 1 (Shim et al.)	59.5	54.8
test 2 (Shim et al.)	40.5	26.2
test 3 (Shim et al.)	48.8	38.1
test 4 (Shim et al.)	48.8	54.9
test 5	119	107.1
test 6	88.1	78.6

#### Example 8: Comparison of Treatment Of Aerobic and Anaerobic Bacteria Using Ammonium Carbamate and Ammonium Carbonate.

Biocides were prepared from sodium hypochlorite and either ammonium carbamate or ammonium carbonate in the presence and absence of sodium bromide, as described below, and immediately added to the samples to be treated. MOs were grown for 48 hours prior to addition of biocide.

Ammonium carbonate was diluted in a stream of DI water (11.71 g ammonium carbonate in 100 ml DI water) to a final concentration of 4680 ppm. Sodium hypochlorite was diluted in a second stream of DI water (4200 ppm, 0.46% w/v expressed as total chlorine). As described above, the two streams were mixed in a conduit to form a biocide (2600 ppm as total chlorine) which was immediately added to the test containers.

In an analogous manner, ammonium carbamate was diluted in DI water (11.71 g ammonium carbamate in 100 ml DI water) to a concentration of 4680 ppm, and mixed in a conduit with a dilute solution of sodium hypochlorite (4200 ppm, 0.46% w/v expressed as total chlorine), and the resulting biocide (2600 ppm as total chlorine) was immediately added to the test containers.

ATP was measured 25 minutes and 120 minutes after feeding the biocide. Residual total chlorine was measured 5 minutes after feeding the biocide, and samples for viable counts were taken after 30 minutes contact time.

The tests were repeated, but with mixing of sodium bromide (6200 ppm) with the ammonium carbonate or ammonium carbamate prior to mixing with the sodium hypochlorite.

Counts of ATP, total aerobic bacteria, growth on a high sugar medium, and killing of anaerobic bacteria were measured. The results are presented in Tables 8A-8G.

Table 8A: Comparison of ATP levels (ng/ml) measured after 25 min

treatment	ammonium carbonate	ammonium carbonate + NaBr	ammonium carbamate	ammonium carbamate + NaBr
1.4 ppm	25.87		30.7	
2.8 ppm	20	17.2	19.2	13.5
5.6 ppm	8.8	21.2	10.13	26.7
8.4 ppm	16		6.7	
14 ppm		2.6	2.3	3.33
28 ppm		1.59		1.16
blank	15.6			40

Table 8B: comparison of ATP levels (ng/ml) measured after 120 min – regrowth potential

treatment	ammonium carbonate	ammonium carbonate + NaBr	ammonium carbamate	ammonium carbamate + NaBr
1.4 ppm	89.3		66.7	
2.8 ppm	101.3	109.3	81.33	117.33
5.6 ppm	41.3	29.3	23.3	23.33
8.4 ppm	8.9		2.5	
14 ppm		1.43	0.77	1.05
28 ppm		0.47		0.22
blank	94.7			110.7

Table 8C: comparison of ATP levels (rlu) measured after 120 minutes

treatment	ammonium carbonate	ammonium carbonate + NaBr	ammonium carbamate	ammonium carbamate + NaBr
1.4 ppm	335000		250000	
2.8 ppm	380000	410000	305000	440000
5.6 ppm	155000	110000	87500	87500
8.4 ppm	33500		9250	
14 ppm		5350	2900	3950
28 ppm		1750		840
blank	355000			415000

Table 8D: comparison of total aerobic bacteria count, cfu/ml after 30 min contact time

treatment	ammonium carbonate	ammonium carbonat + NaBr	ammonium carbamate	ammonium carbamate + NaBr
1.4 ppm	$3.00 \times 10^8$		$5.00 \times 10^7$	
2.8 ppm	$5.00 \times 10^7$	$2.70 \times 10^7$	$1.10 \times 10^7$	$2.40 \times 10^7$
5.6 ppm	$5.00 \times 10^6$	$9.44 \times 10^6$	$7.60 \times 10^6$	$3.20 \times 10^6$
8.4 ppm	$4.00 \times 10^6$		$6.60 \times 10^4$	
14 ppm		$3.20 \times 10^5$	$3.60 \times 10^4$	$2.80 \times 10^5$
28 ppm		$4.40 \times 10^4$		$4.16 \times 10^4$
blank	$4.80 \times 10^7$	$4.80 \times 10^7$	$4.60 \times 10^7$	$4.60 \times 10^7$

Table 8E: comparison of growth on a high sugar medium (cfu/ml), after 30 min contact time

treatment	ammonium carbonate	ammonium carbonate + NaBr	ammonium carbamate	ammonium carbamate + NaBr
1.4 ppm	$3.00 \times 10^7$		$3.00 \times 10^7$	
2.8 ppm	$3.00 \times 10^7$	$1.22 \times 10^5$	$1.10 \times 10^5$	$4.00 \times 10^3$
5.6 ppm	$3.00 \times 10^7$	$1.80 \times 10^4$	$1.00 \times 10^2$	$1.00 \times 10^3$
8.4 ppm	$3.00 \times 10^4$		$1.00 \times 10^1$	
14 ppm		$2.00 \times 10^2$	$1.00 \times 10^1$	$1.00 \times 10^1$
28 ppm		$2.00 \times 10^2$		$2.00 \times 10^1$
blank	$5.00 \times 10^7$			$3.00 \times 10^8$

Table 8F: total anaerobic counts (cfu/ml), after 30 min contact time

treatment	ammonium carbonate	ammonium carbonate + NaBr	ammonium carbamate	ammonium carbamate + NaBr
1.4 ppm	$3.00 \times 10^7$			
2.8 ppm	$2.00 \times 10^6$	$1.00 \times 10^4$	$3.00 \times 10^7$	$1.00 \times 10^3$
5.6 ppm	$5.00 \times 10^6$	$2.10 \times 10^4$	$3.40 \times 10^4$	$1.00 \times 10^3$
8.4 ppm	$2.00 \times 10^3$		$3.00 \times 10^3$	
14 ppm		$1.00 \times 10^2$	$1.00 \times 10^1$	$2.00 \times 10^2$
28 ppm		$1.00 \times 10^2$	$1.00 \times 10^1$	$1.00 \times 10^2$
blank	$3.00 \times 10^7$			$3.00 \times 10^7$

Table 8G: comparison of kill of anaerobic bacteria (cfu), 30 minutes

treatment	ammonium carbonate	ammonium carbonate + NaBr	ammonium carbamate	ammonium carbamate + NaBr
1.4 ppm	$3.00 \times 10^7$		$3.00 \times 10^7$	
2.8 ppm	$2.00 \times 10^6$	$1.00 \times 10^4$	$3.40 \times 10^4$	$1.00 \times 10^3$
5.6 ppm	$5.00 \times 10^6$	$2.10 \times 10^4$	$3.00 \times 10^3$	$1.00 \times 10^3$
8.4 ppm	$2.00 \times 10^3$		10.00	
14 ppm		$1.00 \times 10^2$	10.00	$2.00 \times 10^2$
28 ppm		$1.00 \times 10^2$		$1.00 \times 10^2$
blank	$3 \times 10^7$			$3 \times 10^7$

Example 9: differences in biocides produced using sodium hypochlorite and concentrated or dilute 5,5-dimethyl hydantoin.

Test 15: 10 ml of a stock solution of 5,5-dimethyl hydantoin (DMH) (19.16% w/v) was mixed with 10 ml of concentrated NaOCl solution (11.6% w/v). The resulting biocide was diluted 25-fold to a final concentration of 2320 ppm (expressed as Cl<sub>2</sub>).

The resulting biocide was immediately added to a medium containing calcium hydroxide (663 ppm), sodium bicarbonate (399.7 ppm), and 18% starch (w/v).

Test 17: Test 15 was repeated, but this time the DMH and NaOCl solutions were diluted prior to mixing (DMH: 4 ml, 19.16% w/v was mixed in 100 ml DI water; NaOCl: 4 ml of 11.6 % w/v was mixed in 100 ml DI water; the two diluted solutions were then mixed as described above and the resulting biocide added to the treated sample).

The effects of the biocides on different aspects of MO growth, as well as residual total chlorine, were measured as described in previous examples. Results are shown in Tables 9A-9D.

Table 9A

Kill of aerobic MO's and MO's growing in high glucose medium ("slime")				
	conc. DMH	conc. DMH	dilute DMH	dilute DMH
treatment	aerobic cfu/ml	slime cfu/ml	aerobic cfu/ml	slime cfu/ml
1.16 ppm	$1.02 \times 10^4$	$6.40 \times 10^3$		
2.1 ppm			$9.80 \times 10^3$	$2.60 \times 10^2$
2.32 ppm	$1.00 \times 10^4$			
4.2 ppm			$1.96 \times 10^4$	$4.00 \times 10^2$
4.64 ppm	$1.69 \times 10^3$	$5.00 \times 10^1$		
6.3 ppm			$1.53 \times 10^5$	$1.33 \times 10^3$
23.2 ppm	$1.00 \times 10^0$	$1.00 \times 10^0$		
blank	$8.00 \times 10^3$	$5.00 \times 10^3$	$4.56 \times 10^4$	$1.06 \times 10^4$

Table 9B

Effect of dilute biocide from DMH and NaOCl in the presence and absence of NaBr				
	dilute DMH	dilute DMH + Br <sup>-</sup>	dilute DMH	dilute DMH + Br <sup>-</sup>
treatment	aerobic cfu/ml	aerobic cfu/ml	slime cfu/ml	slime cfu/ml
2.1 ppm	$9.80 \times 10^3$	$8.48 \times 10^4$	$2.60 \times 10^2$	$8.80 \times 10^2$
4.2 ppm	$1.96 \times 10^4$	$1.12 \times 10^4$	$4.00 \times 10^2$	$8.00 \times 10^1$
6.3 ppm	$1.53 \times 10^5$	$1.08 \times 10^4$	$1.33 \times 10^3$	$9.40 \times 10^2$
blank	$4.56 \times 10^4$	$4.56 \times 10^4$	$1.06 \times 10^4$	$1.06 \times 10^4$

Table 9C:

Residual total Cl <sub>2</sub> in water containing a high organic load. Results presented as % of feed rate, ppm as total chlorine.		
dosage (ppm)	Test 15	Test 17
1.16	0	
2.1		42.9
2.32	12.07	
4.2		78.6
4.64	34.05	
6.3		82.5
8.4		
12.6		
23.2	57.33	



The results in Table 9C show the biocide produced by diluting DMH and NaOCl prior to mixing is more stable than biocide produced by mixing concentrated DMH and NaOCl and subsequently diluting.

The ability of biocides of Tests 15 and 17 to control the growth of microorganisms grown on a high sugar medium was compared. The results are presented in Table 9D as % surviving bacteria after exposure for 30 minutes at the indicated concentration.

Table 9D

dosage (ppm)	Test 15 - % survival, 30 min contact time	Test 17 - % survival, 30 min contact time
0	100	100
1.16	100	
2.1		8.3
2.32	9.38	
4.2		0.8
4.64	0.78	
6.3		0.9
8.4		
12.6		
23.2	0.02	

The results show that biocide produced from diluted DMH and NaOCl achieves better control of bacteria grown in high sugar medium than does biocide produced from concentrated DMH and NaOCl.

Example 13: Comparison of biocidal properties of biocides prepared from ammonium sulfamate, ammonium sulfate, sulfamic acid and ammonium carbamate.

As described in earlier examples, biocides were prepared as follows:

Test 23: Sulfamic acid (14.62 g/100 ml DI water) was diluted (4 ml to 100 ml DI water) and  $\text{NH}_3$  (25% w/v in water) was added (0.5 ml). NaOCl (14% as  $\text{Cl}_2$ , 4 ml/100 ml DI water) was mixed with the diluted sulfamic acid.

Test 24: Ammonium sulfate (19.8 g/100 ml DI water) was diluted (2 ml/100 ml DI water). NaOCl (14% as  $\text{Cl}_2$ ) was diluted in DI water (4 ml/100 ml), and mixed with the diluted ammonium sulfate solution.

Test 25: Sulfamic acid (14.62gr/100ml), was mixed with NaOCl (4ml/100ml, 14% as  $\text{Cl}_2$ ).

Test 26: A stock solution of ammonium carbamate (11.55 g/100 ml DI water) was diluted in DI water (4 ml/100ml). NaOCl (14% as  $\text{Cl}_2$ ) was diluted in DI water (4 ml/100 ml). The 2 diluted solutions were mixed.

In tests 23-26, the resulting biocide was immediately added to water containing MOs from pink slime, as described above, and the total residual chlorine in the treated water/medium was measured after 5 minutes and 12 hours. Results are presented in Tables 10A and 10B.

Table 10A: Total residual chlorine after 5 minutes (ppm):

	5 min	5 min	5 min	5 min
feed as Cl <sub>2</sub> (ppm)	H <sub>2</sub> NSO <sub>3</sub> NH <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> NSO <sub>3</sub> H	H <sub>2</sub> NCO <sub>2</sub> NH <sub>4</sub>
1.4 (control - DI water only)	1.4	1.6	0.9	1.2
1.4	0	0	0.3	0
2.8	1.3	0.9	0.7	0.2
7	4.9	5	4	1.3
14	10.7	8.1	10.7	10.2

Table 10B: Total residual chlorine after 12 hours (ppm):

	12 hours	12 hours	12 hours	12 hours
feed as Cl <sub>2</sub> (ppm)	H <sub>2</sub> NSO <sub>3</sub> NH <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> NSO <sub>3</sub> H	H <sub>2</sub> NCO <sub>2</sub> NH <sub>4</sub>
1.4 (control - DI water only)	1.1	1.1	0.9	1.2
1.4	0	0	0.3	0
2.8	0.1	0	0.3	0.2
7	1.1	1.2	2.9	1.3
14	4.1	3.8	9.2	3.9

The results in Tables 10A and 10B show that the biocides derived from sulfamic acid and from ammonium sulfamate were the most stable biocides after 5 minutes. The biocide derived from sulfamic acid remained stable and exhibited high residual total chlorine after 12 hours.

ATP counts for MOs growing on medium treated with the biocides produced in Tests 23-26 were obtained 30 minutes and 12 hours after addition of biocide to the growth medium. The results are shown in Tables 10C and 10D.

Table 10C: ATP measured 20 minutes after feeding the biocide (rlu).

	ATP-20 min	ATP-20 min	ATP-20 min	ATP-20 min
feed as Cl <sub>2</sub> (ppm)	H <sub>2</sub> NSO <sub>3</sub> NH <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> NSO <sub>3</sub> H	H <sub>2</sub> NCO <sub>2</sub> NH <sub>4</sub>
1.4	25500	24000	31500	39000
2.8	19500	28500	26000	16500
7	9950	16000	26000	14000
14	5200	2850	12000	4500
blank	24500	20500	37000	29000

Table 10D: ATP measured 12 hours after feeding the biocide.

	ATP-12 h	ATP-12 h	ATP-12 h	ATP-12 h
feed as Cl <sub>2</sub> (ppm)	H <sub>2</sub> NSO <sub>3</sub> NH <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> NSO <sub>3</sub> H	H <sub>2</sub> NCO <sub>2</sub> NH <sub>4</sub>
1.4	90000	94500	83000	87500
2.8	8550	6000	76000	3950
7	435	460	42000	560
14	380	390	14500	300
blank	87500	90000	95500	95500

Conclusions: at a feed rate of 1.4 ppm, no control was achieved, and the MO's continued to grow. A feed rate of 2.8 ppm as total chlorine was ineffective for biocide formed from sulfamic acid, despite the higher residual left in the process water.

Better control was achieved with ammonium sulfate compared to sodium sulfamate, and still better control with ammonium carbamate after 30 minutes as well as after 12 hours.

Counts of aerobic, anaerobic and high-sugar MOs (cfu/ml) were measured 30 minutes after application of the biocides of Tests 23-26. The results are presented in Tables 10C-10E.

Table 10C: Effect of biocides on growth of aerobic MOs 30 minutes after application.

	aerobic MOs (cfu/ml), 30 minutes			
feed as Cl <sub>2</sub> (ppm)	H <sub>2</sub> NSO <sub>3</sub> NH <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> NSO <sub>3</sub> H	H <sub>2</sub> NCO <sub>2</sub> NH <sub>4</sub>
1.4	1.29 x 10 <sup>6</sup>	1.40 x 10 <sup>6</sup>	1.08 x 10 <sup>6</sup>	9.70 x 10 <sup>5</sup>
2.8	6.16 x 10 <sup>5</sup>	6.40 x 10 <sup>5</sup>	5.40 x 10 <sup>5</sup>	8.96 x 10 <sup>5</sup>
7	4.00 x 10 <sup>5</sup>	3.60 x 10 <sup>5</sup>	8.08 x 10 <sup>5</sup>	5.84 x 10 <sup>5</sup>
14	2.40 x 10 <sup>5</sup>	1.80 x 10 <sup>5</sup>	7.36 x 10 <sup>5</sup>	7.50 x 10 <sup>4</sup>
blank	1.20 x 10 <sup>6</sup>	1.44 x 10 <sup>6</sup>	1.10 x 10 <sup>6</sup>	1.34 x 10 <sup>6</sup>

Table 10D:

Effect of biocides on growth of anaerobic MOs 30 minutes after application.

	anaerobic MOs (cfu/ml), 30 minutes			
feed as Cl <sub>2</sub> (ppm)	H <sub>2</sub> NSO <sub>3</sub> NH <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> NSO <sub>3</sub> H	H <sub>2</sub> NCO <sub>2</sub> NH <sub>4</sub>
1.4	1.50 x 10 <sup>3</sup>	1.00 x 10 <sup>1</sup>	2.50 x 10 <sup>3</sup>	1.00 x 10 <sup>1</sup>
2.8	1.00 x 10 <sup>1</sup>	1.00 x 10 <sup>1</sup>	1.00 x 10 <sup>1</sup>	1.00 x 10 <sup>1</sup>
7	1.00 x 10 <sup>1</sup>	1.00 x 10 <sup>1</sup>	2.00 x 10 <sup>2</sup>	1.00 x 10 <sup>1</sup>
14	1.00 x 10 <sup>1</sup>	1.00 x 10 <sup>1</sup>	3.00 x 10 <sup>2</sup>	1.00 x 10 <sup>1</sup>
blank	1.00 x 10 <sup>3</sup>	1.00 x 10 <sup>3</sup>	1.00 x 10 <sup>3</sup>	1.00 x 10 <sup>3</sup>

Table 10E:

Effect of biocides on growth of high-sugar MOs 30 minutes after application.

	high sugar MOs (cfu/ml), 30 min			
feed as Cl <sub>2</sub> (ppm)	H <sub>2</sub> NSO <sub>3</sub> NH <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> NSO <sub>3</sub> H	H <sub>2</sub> NCO <sub>2</sub> NH <sub>4</sub>
1.4	6.24 x 10 <sup>4</sup>	1.03 x 10 <sup>5</sup>	6.40 x 10 <sup>4</sup>	1.79 x 10 <sup>5</sup>
2.8	5.00 x 10 <sup>2</sup>	4.00 x 10 <sup>2</sup>	3.32 x 10 <sup>4</sup>	2.00 x 10 <sup>2</sup>
7	1.00 x 10 <sup>1</sup>	1.00 x 10 <sup>1</sup>	8.72 x 10 <sup>4</sup>	1.00 x 10 <sup>1</sup>
14	1.00 x 10 <sup>1</sup>	1.00 x 10 <sup>1</sup>	7.30 x 10 <sup>3</sup>	1.00 x 10 <sup>1</sup>
blank	1.20 x 10 <sup>5</sup>	1.10 x 10 <sup>5</sup>	7.00 x 10 <sup>4</sup>	1.10 x 10 <sup>5</sup>

The results shown in Tables 10C-10E clearly show differences in viable counts after a contact time of 30 minutes. At 14 ppm, biocide produced from ammonium carbamate was superior to the other biocides tested.

## Claims

1. A method for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient, the method comprising:

producing a predetermined first dilution of a hypochlorite oxidant;

producing a predetermined second dilution comprising an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

-H,  
-COOH,  
-COOR<sup>3</sup> wherein R<sup>3</sup> is selected from the group consisting of  
C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,  
-CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
-COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,  
-COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,  
-C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
-OH, -COOH, or -NH<sub>2</sub>,  
-SO<sub>3</sub>H,  
-SO<sub>2</sub>R<sup>7</sup> wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,  
-OH,  
-C<sub>1-8</sub> alkyl,  
-C<sub>1-8</sub> alkyl optionally substituted by one or more of:  
-OH, -COOH, -NH<sub>2</sub> or =NH,  
-NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,  
or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above,  
provided that R<sup>1</sup> and R<sup>2</sup> are not both H,  
and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

synchronously metering said first dilution and said second dilution into a mixing vessel to continuously mix therein according to a predetermined ratio to produce said active biocidal ingredient having an effective amount of reproducibility, stability and efficacy *in situ* in said mixing vessel;

and continuously injecting said active biocidal ingredient, as it is produced *in situ* in said mixing vessel, directly from said mixing vessel into the liquid being treated.

2. A method according to claim 1, wherein said second dilution further comprises bromide.
3. A method according to claim 2, wherein said second dilution is produced by mixing said bromide and said ammonium salt to form a mixture of bromide and amine, and then diluting said mixture.
4. A method according to claim 2, wherein said second dilution is produced by forming a third dilution of bromide, forming a fourth dilution of ammonium salt, and mixing said third and fourth dilutions to form said second dilution.
5. A method according to claim 1, wherein said ammonium salt is selected from the group consisting of ammonium sulfamate and ammonium carbamate.
6. A method according to claim 2, wherein the bromide is an alkali or alkaline earth metal bromide salt or a mixture of alkali or alkaline earth metal bromide salts.
7. A method according to claim 6, wherein the bromide is selected from the group consisting of HBr, LiBr, NaBr, KBr, CaBr<sub>2</sub> and MgBr<sub>2</sub> and mixtures thereof.
8. A method according to claim 7, wherein the bromide comprises a salt selected from the group consisting of sodium bromide and potassium bromide.
9. A method according to claim 8 wherein the bromide comprises NaBr.
10. A method according to claim 2 wherein said second dilution contains bromide and ammonium salt in a molar ratio of between 20:1 and 1:10.
11. A method according to claim 10 wherein said second dilution contains bromide and ammonium salt in a molar ratio of between 2:1 and 1:2.
12. A method according to claim 11 wherein said second dilution contains bromide and ammonium salt in equimolar amounts.
13. A method according to claim 2 wherein the molar ratio of primary amine groups in said ammonium salt to said bromide in said second dilution is between 1:10 and 20:1.
14. A method according to claim 13 wherein the molar ratio of primary amine groups in said ammonium salt to said bromide in said second dilution is between 1:2 and 2:1.
15. A method according to claim 14 wherein the molar ratio of primary amine groups in said ammonium salt to said bromide in said second dilution is 1:1.
16. A method according to claim 1, wherein the total amount of bromide and ammonium salt present in said second dilution is between 10 and 40% w/v.

17. A method according to claim 1, wherein the hypochlorite oxidant is selected from the group consisting of sodium hypochlorite, calcium hypochlorite, magnesium hypochlorite, potassium hypochlorite and lithium hypochlorite and mixtures thereof.
18. A method according to claim 17, wherein the hypochlorite oxidant comprises a hypochlorite selected from the group consisting of sodium hypochlorite and calcium hypochlorite.
19. A method according to claim 18, wherein the hypochlorite oxidant comprises sodium hypochlorite.
20. A method according to claim 1, wherein the molar ratio of primary amine groups in said ammonium salt to hypochlorite oxidant expressed as  $\text{Cl}_2$  is from 2:1 to 1:1.
21. A method according to claim 1, wherein the molar ratio of primary amine groups in said ammonium salt to hypochlorite oxidant expressed as  $\text{Cl}_2$  is 1:1.
22. A method according to claim 1, wherein the concentration of hypochlorite oxidant in said first dilution prior to mixing with said second dilution is between 4 and 20% w/v.
23. A method according to claim 1 wherein said predetermined first dilution is continuously produced immediately before it is synchronously metered into said mixer with said predetermined second dilution.
24. A method according to claim 1 wherein said predetermined second dilution is continuously produced immediately before it is synchronously metered into said mixer with said predetermined first dilution.
25. A method according to claim 1 wherein said biocide, as produced *in situ* in said mixer, has a pH of at least 8.0 before being injected into said liquid to be treated.
26. A method according to claim 1 wherein said biocide, as produced *in situ* in said mixer, has a pH of at least 8.5 before being injected into said liquid to be treated.
27. A method according to claim 1 wherein said biocide, as produced *in situ* in said mixer, has a pH of at least 9.0 before being injected into said liquid to be treated.
28. A method according to claim 26 wherein said biocide, as produced *in situ* in said mixer, has a pH of over 9.5 before being injected into said liquid to be treated.
29. A method according to claim 27, wherein said biocide, as produced *in situ* in said mixer, has a pH of less than 11.5 before being injected into said liquid to be treated.
30. A method according to claim 27, wherein said biocide, as produced *in situ* in said mixer, has a pH of less than 11.0 before being injected into said liquid to be treated.

31. A method according to claim 27, wherein said biocide, as produced *in situ* in said mixer, has a pH of less than 10.5 before being injected into said liquid to be treated.
32. A method according to claim 27, wherein said biocide is injected at a rate to maintain in the biocide a stable pH of at least 8.0 as said biocide is produced.
33. A method according to claim 1 wherein the concentration of the biocide immediately prior to being injected into said liquid to be treated is from 1000 to 10,000 ppm expressed as total chlorine.
34. A method according to claim 1 wherein said liquid to be treated has a pH of between about 5 and about 10.5 before said biocide is injected into said liquid.
35. A method according to claim 34, wherein said liquid to be treated has a pH of between about 7 and about 9 before said biocide is injected into said liquid.
36. A method according to claim 1, wherein said biocide, as produced *in situ* in the mixer, is injected into said liquid to be treated to a concentration of 0.5-300 ppm expressed as chlorine.
37. A method according to claim 36, wherein said biocide, as produced *in situ* in the mixer, is injected into said liquid to be treated to a concentration of 3-10 ppm expressed as chlorine.
38. A method according to claim 1, wherein said liquid to be treated is water.
39. A method according to claim 1, wherein the biocide is effective within 24 hours of injection into the liquid to be treated.
40. A method according to claim 39, wherein the biocide is effective within 1 hour of injection into the liquid to be treated.
41. A method according to claim 39, wherein the biocide is effective within 20 minutes of injection into the liquid to be treated.
42. A method according to claim 1, wherein said biocide is capable of killing at least 50% of the microorganisms in a liquid test sample within 3 hours after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
43. A method according to claim 1, wherein said biocide is capable of killing at least 50% of the microorganisms in a liquid test sample within 1 hour after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
44. A method according to claim 1, wherein said biocide is capable of killing at least 50% of the microorganisms in a liquid test sample within 30 minutes after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.

45. A method according to claim 1, wherein said biocide is capable of killing at least 75% of the microorganisms in a liquid test sample within 3 hours after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
46. A method according to claim 1, wherein said biocide is capable of killing at least 75% of the microorganisms in a liquid test sample within 1 hour after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
47. A method according to claim 1, wherein said biocide is capable of killing at least 75% of the microorganisms in a liquid test sample within 30 minutes after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
48. A method according to claim 1, wherein said biocide is capable of killing at least 90% of the microorganisms in a liquid test sample within 3 hours after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
49. A method according to claim 1, wherein said biocide is capable of killing at least 90% of the microorganisms in a liquid test sample within 1 hour after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
50. A method according to claim 1, wherein said biocide is capable of killing at least 90% of the microorganisms in a liquid test sample within 30 minutes after administration, with a residual of biocide, expressed as total chlorine, of at least 0.5 ppm.
51. A method according to claim 1, wherein said liquid to be treated is present in a system from which a portion of said liquid to be treated is discharged and replaced during the regular course of operation of said system.
52. A method according to claim 51 wherein said system is a paper mill.
53. A method according to claim 52 wherein said liquid is process water.
54. A method according to claim 51 wherein said system is a cooling tower.
55. A method according to claim 51, wherein said portion of said liquid to be treated which is discharged and replaced during the regular course of operation of said system is continuously discharged and replaced during the regular course of operation of said system.
56. A method according to claim 51, wherein said portion of said liquid to be treated which is discharged and replaced during the regular course of operation of said system is discharged and replaced at least once every 24 hours during the regular course of operation of said system.



57. A method for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient, the method comprising:

producing a predetermined first dilution of a hypochlorite oxidant;

producing a predetermined second dilution comprising an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

- H,
- COOH,
- COOR<sup>3</sup> wherein R<sup>3</sup> is selected from the group consisting of C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,
- CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
  - COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,
- COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,
- C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
  - OH, -COOH, or -NH<sub>2</sub>,
- SO<sub>3</sub>H,
- SO<sub>2</sub>R<sup>7</sup> wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,
- OH,
- C<sub>1-8</sub> alkyl,
- C<sub>1-8</sub> alkyl optionally substituted by one or more of:
  - OH, -COOH, -NH<sub>2</sub> or =NH,
- NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,

or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above, provided that R<sup>1</sup> and R<sup>2</sup> are not both H,

and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

synchronously metering said first dilution and said second dilution into a vessel to continuously mix therein according to a predetermined ratio to produce said active biocidal ingredient having an effective amount of reproducibility, stability and efficacy *in situ* in said vessel;

and continuously injecting said active biocidal ingredient, as it is produced *in situ* in said mixing vessel, directly from said mixing vessel into the liquid being treated.

58. A method according to claim 57, further comprising producing a predetermined third dilution comprising bromide, and wherein said synchronously metering comprises synchronously metering said third dilution into said vessel to continuously mix therein with said first dilution and second dilution according to a predetermined ratio to produce said active biocidal having an effective amount of reproducibility, stability and efficacy *in situ* in said vessel.

59. A method according to claim 57, wherein said vessel is a mixing vessel.

60. A method for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient formed by mixing an oxidant comprising hypochlorite, and an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

- H,
  - COOH,
  - COOR<sup>3</sup> wherein R<sup>3</sup> is selected from the group consisting of C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,
  - CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,
  - COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,
  - C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - OH, -COOH, or -NH<sub>2</sub>,
  - SO<sub>3</sub>H
  - SO<sub>2</sub>R<sup>7</sup> wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,
  - OH,
  - NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,
  - C<sub>1-8</sub> alkyl,
  - C<sub>1-8</sub> alkyl optionally substituted by one or more of:
    - OH, -COOH, -NH<sub>2</sub> or =NH,
- or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above, provided that R<sup>1</sup> and R<sup>2</sup> are not both H, and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

the method characterized in:

continuously and synchronously injecting a quantity of said oxidant into a first stream of water passing through a first conduit to produce therein a predetermined dilution of said oxidant;

continuously and synchronously injecting a quantity of said ammonium salt into a second stream of water passing through a second conduit to produce therein a predetermined dilution of said ammonium salt;

continuously and synchronously injecting said first and second streams into a third conduit according to a predetermined ratio to produce said active biocidal ingredient in situ in said third conduit;

and continuously injecting said active biocidal ingredient, as it is produced in situ in said third conduit directly from said third conduit into the liquid being treated.

61. The method according to claim 61, further comprising continuously and synchronously injecting a quantity of a bromide into a third stream of water passing through a fourth conduit to produce therein a predetermined dilution of said bromide;

and wherein said continuously and synchronously injecting said first and second streams into a third conduit comprising continuously and synchronously injecting said first, second and third streams into said third conduit according to a predetermined ratio to produce said active biocidal ingredient in situ in said third conduit.

62. The method according to claim 60, wherein said oxidant is continuously injected into said first stream of water by a first dosing pump connected to reservoir of said oxidant.

63. The method according to claim 61, wherein said ammonium salt and said bromide are provided in a mixture, said second and third streams are the same stream and said second and fourth conduit are the same conduit, and said mixture comprising said bromide and said ammonium salt is continuously injected into said second stream of water by a second dosing pump connected to a reservoir of said mixture of said bromide and said ammonium salt synchronously operated with said first dosing pump.

64. The method according to claim 61, wherein said second and third streams are mixed prior to injecting into said third conduit.

65. The method according to claim 61, wherein said first and third streams are mixed prior to injecting into said third conduit.

66. A method for improving the efficacy of biocide produced from an ammonium salt and a hypochlorite oxidant, the method comprising:

producing a predetermined first dilution of a hypochlorite oxidant;

producing a predetermined second dilution of a mixture comprising bromide and an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

-H,

-COOH,

-COOR<sup>3</sup> wherein  $\text{R}^3$  is selected from the group consisting of

$C_{1-8}$  alkyl,  $C_{3-8}$  cycloalkyl,  $C_{4-9}$  cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,  
 $-CHR^4COOH$  wherein  $R^4$  is selected from the group consisting of H,  $C_{3-8}$  cycloalkyl,  $C_{4-9}$  cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and  $C_{1-8}$  alkyl, wherein  $C_{1-8}$  alkyl is optionally substituted by  
 $-COOH$ ,  $-SCH_3$ ,  $-NH_2$ ,  $-NHC(=NH)NH_2$ ,  $-C(=O)NH_2$ ,  $-SH$ ,  $-OH$ , 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,  
 $-COONHR^5$  wherein  $R^5$  is H,  $C_{1-8}$  alkyl,  $C_{3-8}$  cycloalkyl,  $C_{4-9}$  cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,  
 $-C(=O)-NHR^6$ ,  $-C(=S)-NHR^6$ ,  $-C(=NH)-NHR^6$  or  $-C(=O)-CH_2R^6$ , wherein  $R^6$  is H, phenyl, 4-methylphenyl, or  $C_{1-8}$  alkyl, wherein  $C_{1-8}$  alkyl is optionally substituted by  
 $-OH$ ,  $-COOH$ , or  $-NH_2$ ,  
 $-SO_3H$ ,  
 $-SO_2R^7$  wherein  $R^7$  is H,  $C_{1-8}$  alkyl, phenyl, 4-methylphenyl, or  $-NH_2$ ,  
 $-OH$ ,  
 $-NHR^7$ , wherein  $R^7$  is H,  $C_{1-8}$  alkyl, phenyl, 4-methylphenyl, benzyl or  $-NH_2$ ,  
 $-C_{1-8}$  alkyl,  
 $-C_{1-8}$  alkyl optionally substituted by one or more of:  
 $-OH$ ,  $-COOH$ ,  $-NH_2$  or  $=NH$ ,  
 or one of  $R^1$  and  $R^2$ , together with the nitrogen atom to which they are attached, form a ring, and the other of  $R^1$  and  $R^2$  is as defined above,  
 provided that  $R^1$  and  $R^2$  are not both H,  
 and provided that at least one of  $R^1$  and  $R^2$  comprises a group  $-COOH$  or  $-SO_3H$ ;

synchronously metering said first dilution and said second dilution into a mixing vessel to continuously mix therein according to a predetermined ratio to produce said biocide; and

continuously injecting said biocide, as it is produced *in situ* in said mixing vessel, directly from said mixing vessel into a liquid to be treated with said biocide.

67. A method for improving the efficacy of biocide produced from an ammonium salt and a hypochlorite oxidant, the method comprising:

producing a predetermined first dilution of a hypochlorite oxidant;

producing a predetermined second dilution of bromide;

producing a predetermined third dilution of an ammonium salt of a compound of the formula  $NHR^1R^2$ , wherein  $R^1$  and  $R^2$  are each independently

$-H$ ,

$-COOH$ ,

$-COOR^3$  wherein  $R^3$  is selected from the group consisting of

$C_{1-8}$  alkyl,  $C_{3-8}$  cycloalkyl,  $C_{4-9}$  cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,

-CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
 -COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,  
 -COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,  
 -C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
 -OH, -COOH, or -NH<sub>2</sub>,  
 -SO<sub>3</sub>H,  
 -SO<sub>2</sub>R<sup>7</sup> wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,  
 -OH,  
 -NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,  
 -C<sub>1-8</sub> alkyl,  
 -C<sub>1-8</sub> alkyl optionally substituted by one or more of:  
 -OH, -COOH, -NH<sub>2</sub> or =NH,  
 or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above, provided that R<sup>1</sup> and R<sup>2</sup> are not both H, and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

synchronously metering said first dilution, said second dilution and said third dilution into a mixing vessel to continuously mix therein according to a predetermined ratio to produce said biocide; and

continuously injecting said biocide, as it is produced *in situ* in said mixing vessel, directly from said mixing vessel into a liquid to be treated with said biocide.

68. A method according to claim 67, wherein said second and third dilutions are mixed prior to mixing with said first dilution.

69. A method according to claim 67, wherein said first and third dilutions are mixed prior to mixing with said second dilution.

70. Apparatus for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient formed by continuously and synchronously mixing a first dilution comprising a hypochlorite oxidant and a second dilution comprising an ammonium salt of a compound of the formula NHR<sup>1</sup>R<sup>2</sup>, wherein R<sup>1</sup> and R<sup>2</sup> are each independently

-H,  
 -COOH,  
 -COOR<sup>3</sup> wherein R<sup>3</sup> is selected from the group consisting of  
 C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,

-CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
 -COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,  
 -COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,  
 -C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>-C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
 -OH, -COOH, or -NH<sub>2</sub>,  
 -SO<sub>3</sub>H  
 -SO<sub>2</sub>R<sup>7</sup> wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,  
 -OH,  
 -NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,  
 -C<sub>1-8</sub> alkyl,  
 -C<sub>1-8</sub> alkyl optionally substituted by one or more of:  
 -OH, -COOH, -NH<sub>2</sub> or =NH,  
 or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above, provided that R<sup>1</sup> and R<sup>2</sup> are not both H, and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

the apparatus comprising:

- (a) a first diluter producing a predetermined first dilution of said oxidant;
- (b) a second diluter producing a predetermined second dilution of said ammonium salt;
- (c) a mixing vessel in which said first and second dilutions are continuously and synchronously mixed according to a predetermined ratio to produce said active biocidal ingredient *in situ* in said mixing vessel; and
- (d) an egress from which said active biocidal ingredient is continuously injected, as it is produced *in situ* in said mixing vessel, directly from said mixing vessel into the liquid being treated.

71. Apparatus according to claim 70, wherein said ammonium salt is present in a mixture with a bromide, and said predetermined second dilution of ammonium salt further comprises bromide.

72. Apparatus according to claim 70, wherein said egress comprises a conduit.

73. Apparatus according to claim 70, wherein said oxidant is selected from the group consisting of alkali and alkaline earth metal hypochlorites and mixtures thereof.

74. Apparatus for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient formed by continuously and synchronously mixing a first dilution comprising a hypochlorite oxidant, a second dilution comprising bromide and a third dilution comprising an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

- H,
  - COOH,
  - COOR<sup>3</sup> wherein  $\text{R}^3$  is selected from the group consisting of  
C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,
  - CHR<sup>4</sup>COOH wherein  $\text{R}^4$  is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
-COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,
  - COONHR<sup>5</sup> wherein  $\text{R}^5$  is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,
  - C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein  $\text{R}^6$  is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by  
-OH, -COOH, or -NH<sub>2</sub>,
  - SO<sub>3</sub>H
  - SO<sub>2</sub>R<sup>7</sup> wherein  $\text{R}^7$  is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,
  - OH,
  - NHR<sup>7</sup>, wherein  $\text{R}^7$  is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,
  - C<sub>1-8</sub> alkyl,
  - C<sub>1-8</sub> alkyl optionally substituted by one or more of:  
-OH, -COOH, -NH<sub>2</sub> or =NH,
- or one of  $\text{R}^1$  and  $\text{R}^2$ , together with the nitrogen atom to which they are attached, form a ring, and the other of  $\text{R}^1$  and  $\text{R}^2$  is as defined above, provided that  $\text{R}^1$  and  $\text{R}^2$  are not both H, and provided that at least one of  $\text{R}^1$  and  $\text{R}^2$  comprises a group -COOH or -SO<sub>3</sub>H;

the apparatus comprising:

- (a) a first diluter producing a predetermined first dilution of said oxidant;
- (b) a second diluter producing a predetermined second dilution of said bromide;
- (c) third diluter producing a predetermined third dilution of said ammonium salt;
- (d) a vessel in which said first, second and third dilutions are continuously and synchronously mixed according to a predetermined ratio to produce said active biocidal ingredient *in situ* in said vessel; and

- (e) an egress from which said active biocidal ingredient is continuously injected, as it is produced *in situ* in said vessel, directly from said vessel into the liquid being treated.

75. An apparatus according to claim 74, wherein said vessel is mixing vessel.

76. Apparatus for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient formed by continuously and synchronously mixing a first dilution comprising a hypochlorite oxidant, a second dilution comprising an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

- H,
  - COOH,
  - COOR<sup>3</sup> wherein R<sup>3</sup> is selected from the group consisting of C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,
  - CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,
  - COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,
  - C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - OH, -COOH, or -NH<sub>2</sub>,
  - SO<sub>3</sub>H
  - SO<sub>2</sub>R<sup>7</sup> wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,
  - OH,
  - NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,
  - C<sub>1-8</sub> alkyl,
  - C<sub>1-8</sub> alkyl optionally substituted by one or more of:
    - OH, -COOH, -NH<sub>2</sub> or =NH,
- or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above, provided that R<sup>1</sup> and R<sup>2</sup> are not both H, and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

the apparatus comprising:

- (a) a first diluter producing a predetermined first dilution of said oxidant;
- (b) a second diluter producing a predetermined third dilution of said ammonium salt;



- (c) a vessel in which said first and second dilutions are continuously and synchronously mixed according to a predetermined ratio to produce said active biocidal ingredient *in situ* in said vessel; and
- (d) an egress from which said active biocidal ingredient is continuously injected, as it is produced *in situ* in said vessel, directly from said vessel into the liquid being treated.

77. An apparatus according to claim 76, wherein said vessel is mixing vessel.

78. Apparatus for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient formed by continuously and synchronously mixing a first solution comprising a hypochlorite oxidant and a second solution comprising an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

- H,
  - COOH,
  - COOR<sup>3</sup> wherein R<sup>3</sup> is selected from the group consisting of C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,
  - CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,
  - COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,
  - C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup>, -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - OH, -COOH, or -NH<sub>2</sub>,
  - SO<sub>3</sub>H,
  - SO<sub>2</sub>R<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,
  - OH,
  - NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,
  - C<sub>1-8</sub> alkyl,
  - C<sub>1-8</sub> alkyl optionally substituted by one or more of:
    - OH, -COOH, -NH<sub>2</sub> or =NH,
- or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above, provided that R<sup>1</sup> and R<sup>2</sup> are not both H, and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

the apparatus comprising:

- (a) a first diluter producing a predetermined first dilution of said first solution;

- (b) a second diluter producing a predetermined second dilution of said second solution;
- (c) a mixing vessel in which said first and second dilutions are continuously and synchronously mixed according to a predetermined ratio to produce said active biocidal ingredient *in situ* in said vessel; and
- (d) an egress from which said active biocidal ingredient is continuously injected, as it is produced *in situ* in said mixing vessel, directly from said mixing vessel into the liquid being treated.

79. Apparatus according to claim 78, wherein said second solution further comprises bromide.

80. Apparatus for controlling microbial fouling in a liquid by adding to the liquid an active biocidal ingredient formed by continuously and synchronously mixing a first solution comprising a hypochlorite oxidant, a second solution comprising a bromide, and a third solution comprising an ammonium salt of a compound of the formula  $\text{NHR}^1\text{R}^2$ , wherein  $\text{R}^1$  and  $\text{R}^2$  are each independently

- H,
  - COOH,
  - COOR<sup>3</sup> wherein R<sup>3</sup> is selected from the group consisting of C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl and benzyl,
  - CHR<sup>4</sup>COOH wherein R<sup>4</sup> is selected from the group consisting of H, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, benzyl, and C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - COOH, -SCH<sub>3</sub>, -NH<sub>2</sub>, -NHC(=NH)NH<sub>2</sub>, -C(=O)NH<sub>2</sub>, -SH, -OH, 4-hydroxyphenyl, 5-imidazolyl, or 3-indolyl,
  - COONHR<sup>5</sup> wherein R<sup>5</sup> is H, C<sub>1-8</sub> alkyl, C<sub>3-8</sub> cycloalkyl, C<sub>4-9</sub> cycloalkylalkyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl, or benzyl,
  - C(=O)-NHR<sup>6</sup>, -C(=S)-NHR<sup>6</sup> -C(=NH)-NHR<sup>6</sup> or -C(=O)-CH<sub>2</sub>R<sup>6</sup>, wherein R<sup>6</sup> is H, phenyl, 4-methylphenyl, or C<sub>1-8</sub> alkyl, wherein C<sub>1-8</sub> alkyl is optionally substituted by
    - OH, -COOH, or -NH<sub>2</sub>,
  - SO<sub>3</sub>H,
  - SO<sub>2</sub>R<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, or -NH<sub>2</sub>,
  - OH,
  - NHR<sup>7</sup>, wherein R<sup>7</sup> is H, C<sub>1-8</sub> alkyl, phenyl, 4-methylphenyl, benzyl or -NH<sub>2</sub>,
  - C<sub>1-8</sub> alkyl,
  - C<sub>1-8</sub> alkyl optionally substituted by one or more of:
    - OH, -COOH, -NH<sub>2</sub> or =NH,
- or one of R<sup>1</sup> and R<sup>2</sup>, together with the nitrogen atom to which they are attached, form a ring, and the other of R<sup>1</sup> and R<sup>2</sup> is as defined above, provided that R<sup>1</sup> and R<sup>2</sup> are not both H,

and provided that at least one of R<sup>1</sup> and R<sup>2</sup> comprises a group -COOH or -SO<sub>3</sub>H;

the apparatus comprising:

- (a) a first diluter producing a predetermined first dilution of said first solution;
  - (b) a second diluter producing a predetermined second dilution of said second solution;
  - (c) a third diluter producing a predetermined third dilution of said third solution;
  - (d) a vessel in which said first, second and third dilutions are continuously and synchronously mixed according to a predetermined ratio to produce said active biocidal ingredient *in situ* in said vessel; and
  - (e) an egress from which said active biocidal ingredient is continuously injected, as it is produced *in situ* in said vessel, directly from said vessel into the liquid being treated.
81. An apparatus according to claim 80, wherein said vessel is a mixing vessel.
82. As a composition of matter, monochloro ammonium carbamate.
83. Water containing monochloro ammonium carbamate.

FIG. 1

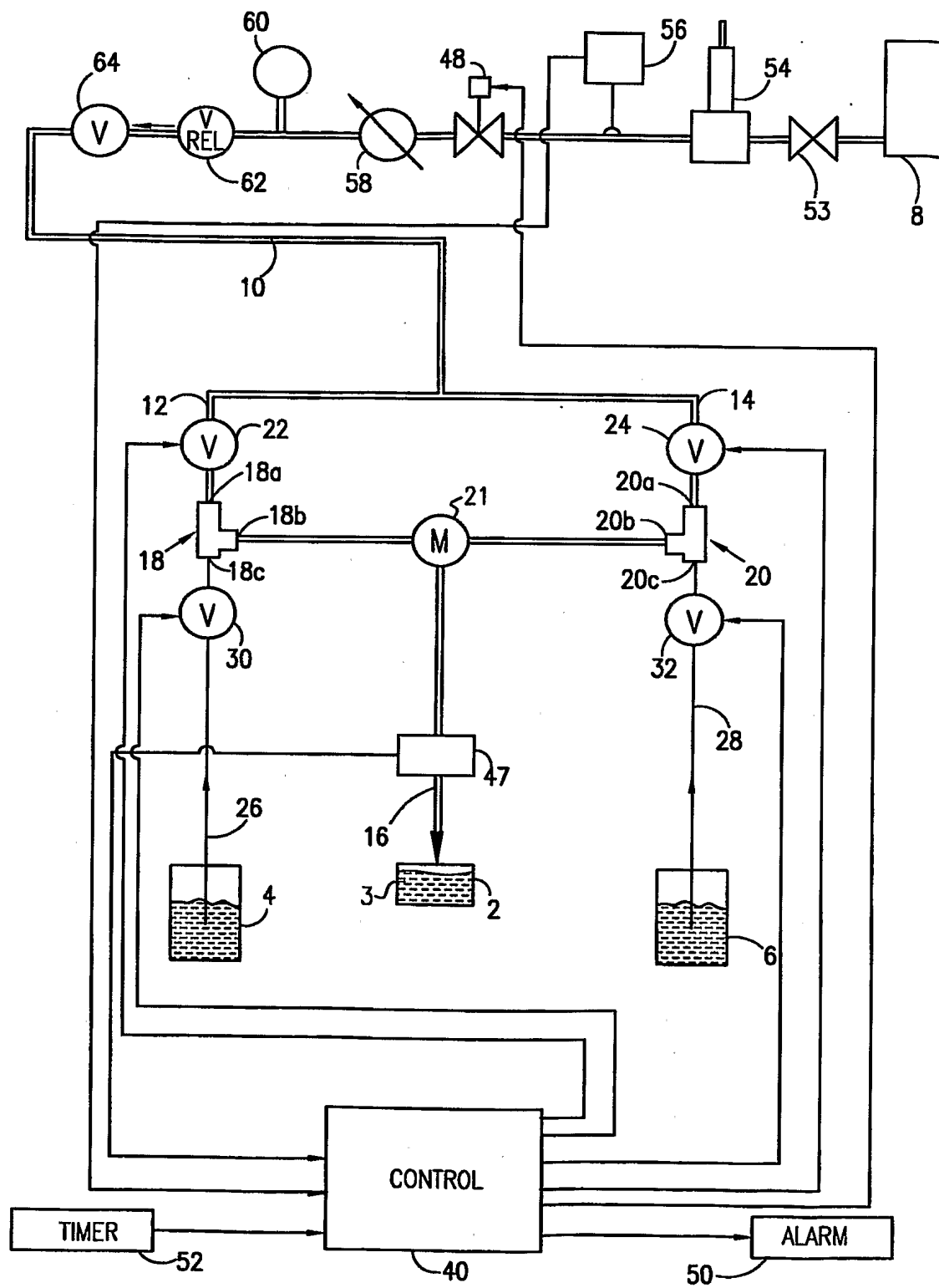


FIG. 2

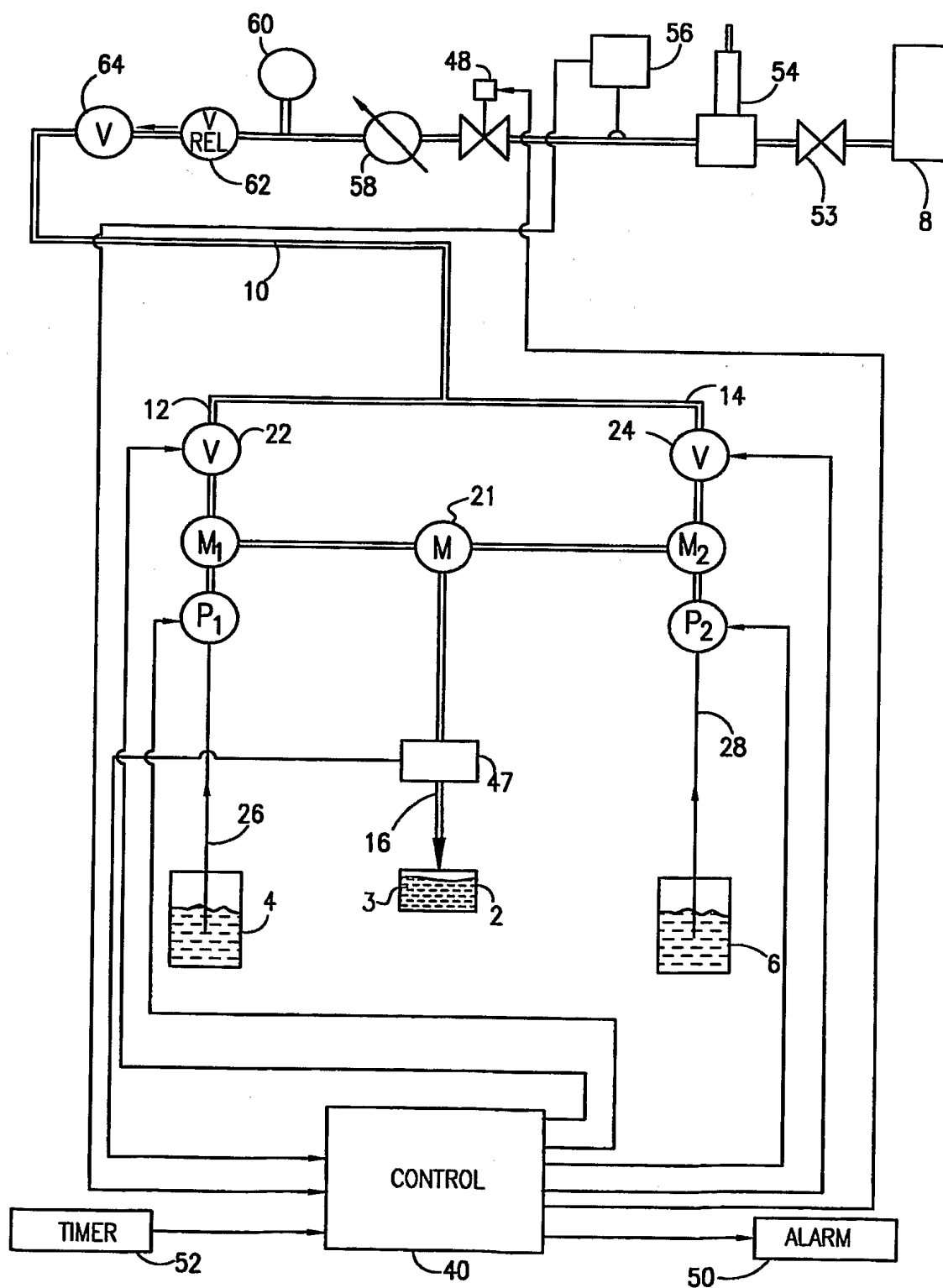


FIG. 3

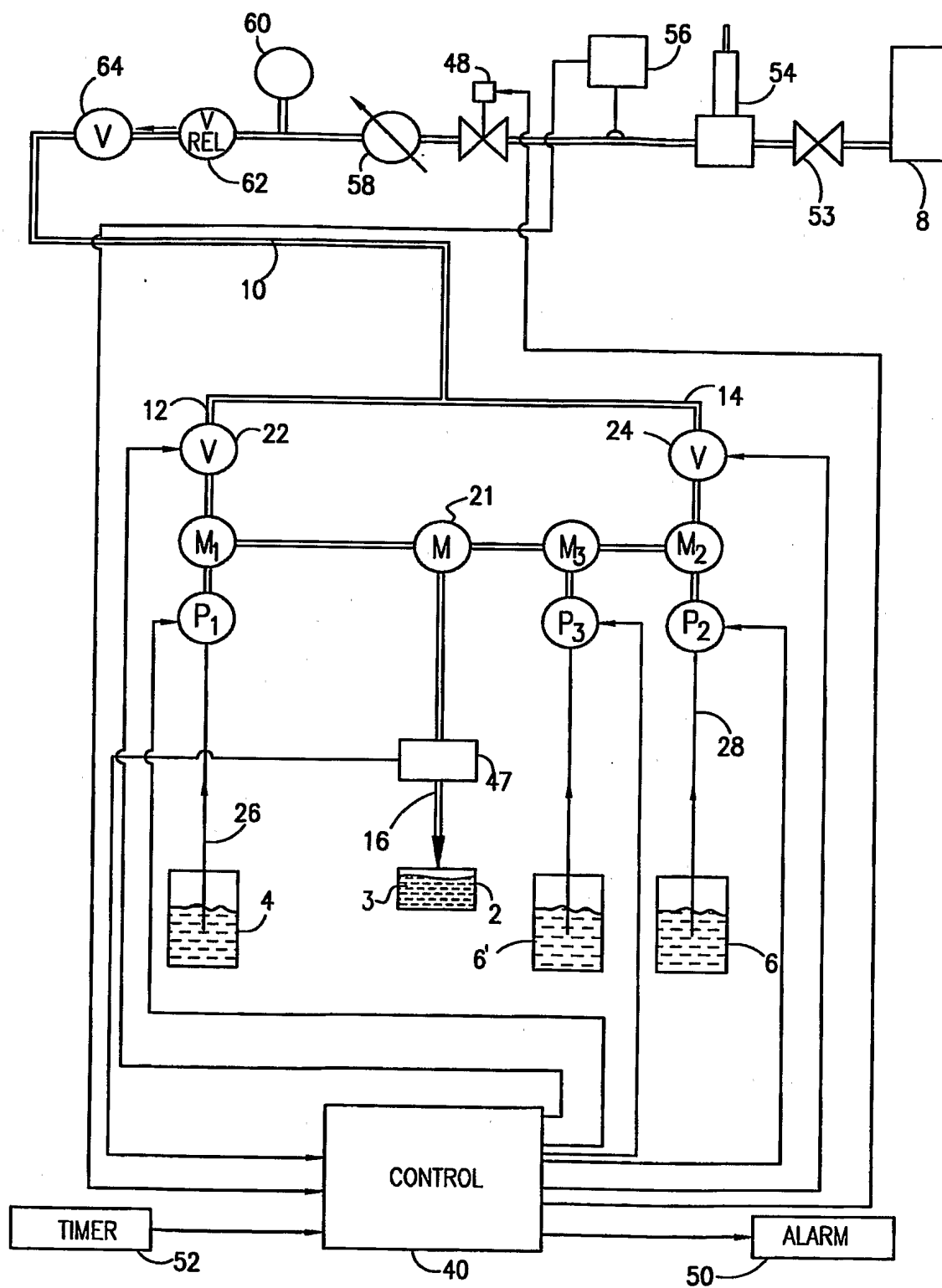


FIG. 4

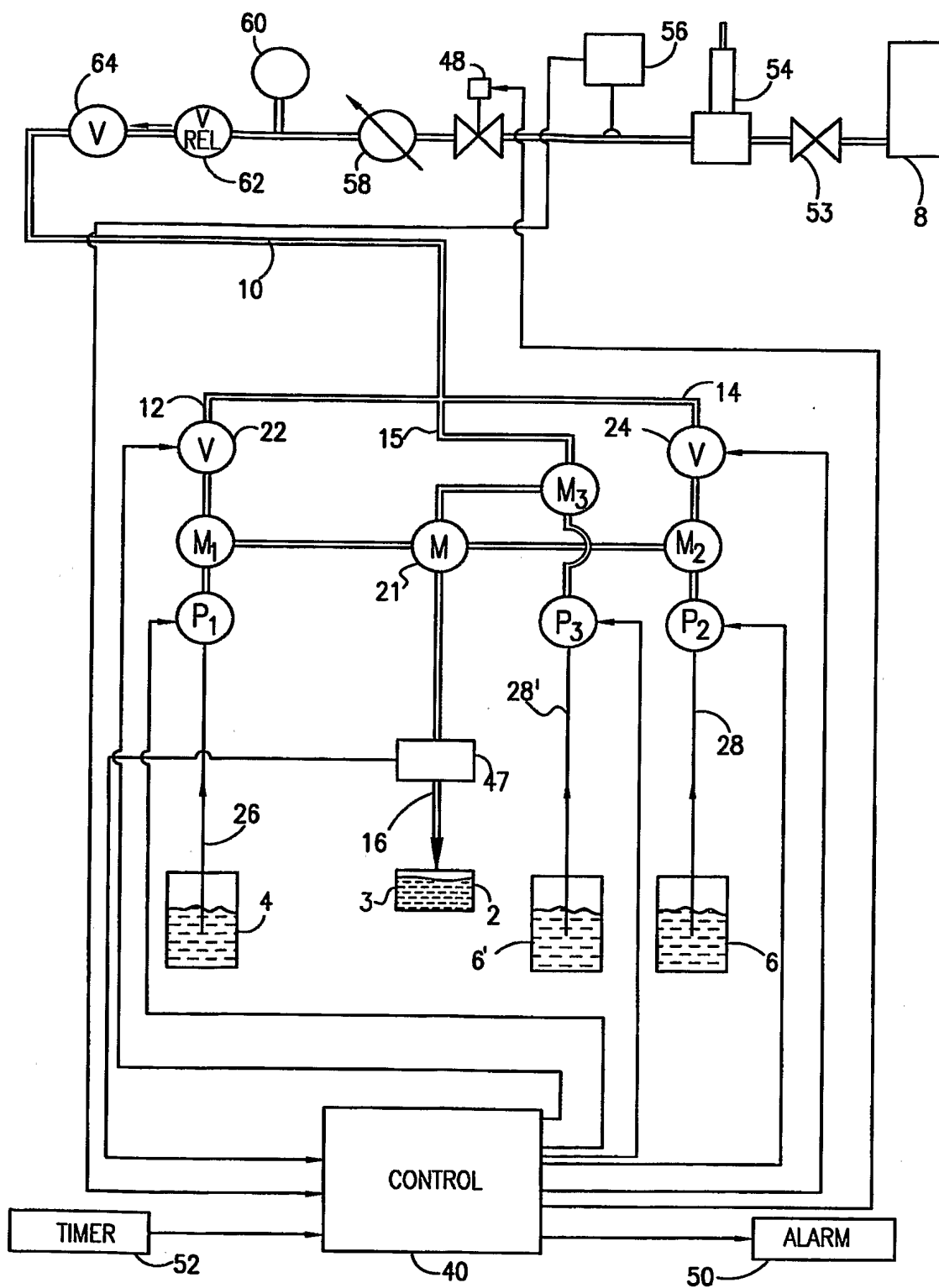


FIG. 5

